Available Online: 27 October 2025 DOI: 10.54254/3029-0821/2025.28377

Analysis of lysine-targeted covalent inhibitors for GSTO1

Ye Fang

School of Pharmacy, Fudan University, Shanghai, China

24301030022@m.fudan.edu.cn

Abstract. This study focuses on lysine-targeted covalent inhibitors of Glutathione S-Transferase Omega 1 (GSTO1). As a member of the glutathione enzyme family, GSTO1 is mainly involved in Phase II detoxification. Its expression is upregulated in various cancers, enhancing the drug resistance of cancer cells, thus making it a potential target for cancer therapy. Inhibiting GSTO1 can suppress the proliferation of tumor cells and promote their apoptosis without affecting the growth of normal cells. Therefore, we can select a small-molecule covalent inhibitor to inhibit the activity of GSTO1. Covalent small-molecule inhibitors have attracted significant attention due to their high selectivity and long-lasting efficacy. Currently, the cysteine-targeted inhibitors under research suffer from short half-lives and poor stability, making it difficult to achieve breakthroughs in clinical applications. Future research should focus on targeting lysine and employ a variety of technical approaches to develop highly active and stable lysine-targeted covalent small-molecule inhibitors for GSTO1, with the aim of achieving breakthroughs in cancer treatment.

Keywords: lysine, GSTO1, covalent inhibitor

1. Introduction

Nowadays, drug therapy for cancer still faces numerous challenges such as drug resistance and poor targeting. The search for new targets and effective inhibitors is extremely urgent. Glutathione S-Transferase Omega 1 (GSTO1), a member of the Glutathione S-Transferase (GST) family, exhibits abnormally upregulated expression in various cancers and is closely associated with increased drug resistance of cancer cells, making it a highly promising target for cancer treatment. Covalent inhibitors have gained much attention in the field of drug development due to their high selectivity and long-lasting efficacy. Lysine, a common amino acid in proteins, has a side-chain amino group that provides possibilities for the development of covalent ligands. This study aims to conduct an in-depth exploration of lysine-targeted covalent inhibitors for GSTO1, providing a new strategy for cancer treatment.

2. Introduction to GSTO1

2.1. Metabolic function of GSTO1

Glutathione S-Transferase Omega 1 (GSTO1) is a member of the extensive Glutathione S-Transferase (GST) family. The main function of GSTO1 is to catalyze the binding of Glutathione (GSH) to a variety of electrophilic compounds, forming complexes, i.e., participating in Phase II detoxification through GSH conjugation [1]. Glutathione can also bind to cysteine residues in proteins, leading to the glutathionylation of proteins to regulate their functions [2, 3].

As an atypical GST isoform, GSTO1 contains a cysteine residue in its active site instead of tyrosine, which is commonly found in other GSTs. This structural difference endows GSTO1 with distinct enzymatic and metabolic functions. It has been observed that the expression of GSTO1 is upregulated in cancers such as esophageal squamous cell carcinoma, pancreatic cancer, transitional cell carcinoma, breast cancer, and colorectal cancer, thereby enhancing the drug resistance of these cancer cells. For example, GSTO1 is one of the key metabolic enzymes required for EGFR-TKI resistance in lung adenocarcinoma cells. Clinical data indicate that the level of GSTO1 is positively correlated with the level of NPM1, NF-κB-mediated transcription, and the progression of human lung adenocarcinoma [4]. Figure 1 illustrates the mechanisms of EGFR-TKI resistance.

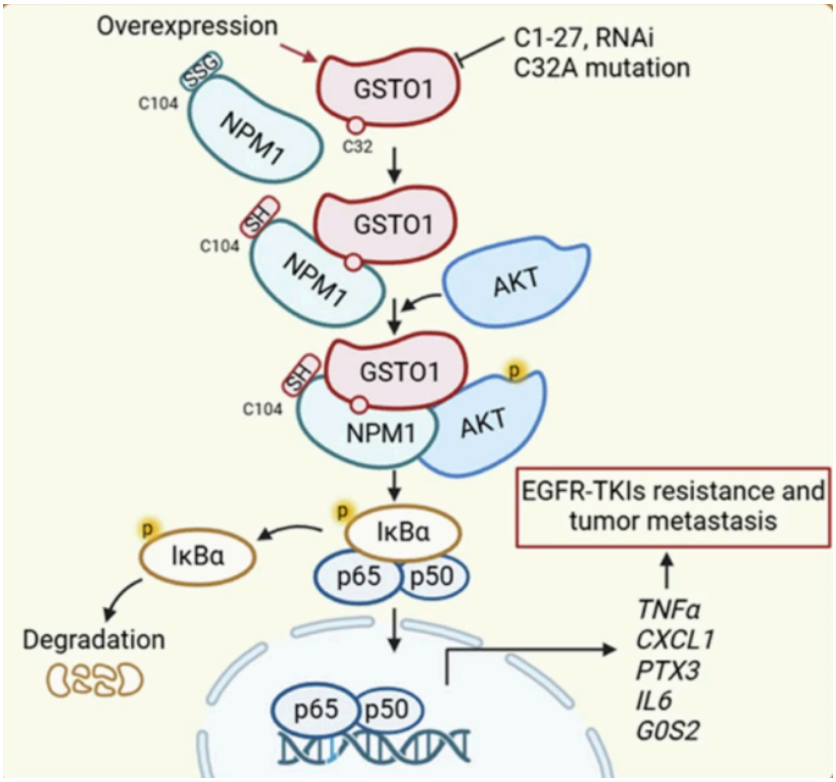

Figure 1. Mechanism of EGFR-TKI resistance

Therefore, inhibiting the function of GSTO1 can play a certain auxiliary role in the treatment of several types of cancers. Consequently, researchers undoubtedly expect that the proliferation and growth of cancer cells lacking GSTO1 will be inhibited, while the proliferation and growth of normal cells lacking GSTO1 will remain normal.

2.2. Effects of GSTO1 deficiency on cells

2.2.1. Growth of tumor cells with GSTO1 deficiency

There are multiple methods to create an equivalent state of tumor cells with GSTO1 deficiency. Researchers can choose to inhibit the expression of the GSTO1 gene in tumor cells, such as through gene silencing. Studies have shown that Glioblastoma Multiforme (GBM) is the most malignant type of glioma and a form of cancer cells. There is sufficient evidence that N6-methyladenosine (m6A) is involved in the progression of GBM, in which the expression of Fat Mass and Obesity-associated protein (FTO), a demethylase that is downregulated. Overexpression of FTO can inhibit cell proliferation and promote cell apoptosis in vitro. In addition, the expression of GSTO1 is upregulated in GBM. Inhibiting the expression of GSTO1 can suppress cell proliferation, promote cell apoptosis, and induce oxidative stress. Furthermore, FTO inhibits the m6A methylation of GSTO1, reducing the stability of GSTO1. Overexpression of GSTO1 eliminates the FTO-mediated processes in T98G cells. In vivo experiments have demonstrated that FTO inhibits tumor growth by downregulating the expression of GSTO1.

In conclusion, FTO induces cell apoptosis by inhibiting the m6A methylation of GSTO1, thereby slowing down the progression of GBM. Inhibiting the expression of GSTO1 can suppress the viability of T98G cells and reduce their number. Moreover, silencing of GSTO1 promotes the death of T98G cells. To sum up, the expression of GSTO1 is upregulated in GBM, and knockdown of GSTO1 inhibits cell proliferation and induces cell apoptosis [5]. Figure 2 shows the conditions related to the colony formation assay.

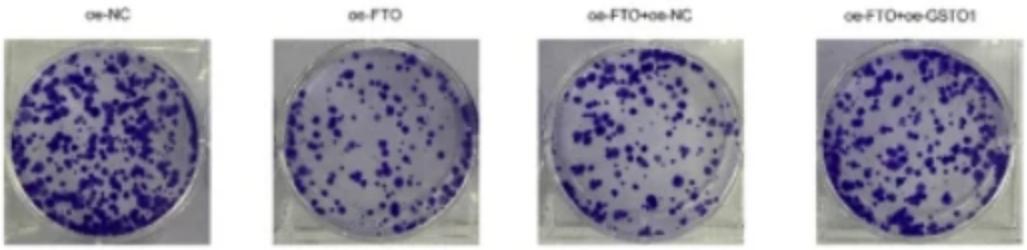

Figure 2. Colony formation assay for evaluating colony-forming ability

Therefore, inhibition or deficiency of GSTO1 helps to inhibit the proliferation of cancer cells.

2.2.2. Growth of normal cells with GSTO1 deficiency

From the previous studies, it can be found that the proliferation of cancer cells with inhibited GSTO1 is indeed suppressed. However, whether pharmacologically induced GSTO1 deficiency affects the normal growth of normal cells is also an issue that needs to be investigated.

In fact, in previous studies, there have been research on developing biochemical tools for the identification and functional analysis of S-glutathionylation, studying knockout mouse models, and developing and evaluating chemical inhibitors of enzymes involved in glutathionylation [1]. Among these, normal mice with GSTO1 knockout did not show abnormal phenotypes, which indicates that pharmacological GSTO1 knockout does not affect the growth of normal mice.

2.3. Pharmacological rationale for GSTO1 inhibitors

At present, some researchers have verified that GSTO1 can serve as an influential target in oncology. By combining transcriptome analysis with proteomics, they have identified oncology-related cellular pathways and novel pharmacodynamic markers. Undoubtedly, some of these pathways and markers are regulated by GSTO1. In subsequent experiments, they used CRISPR/Cas9 technology to construct the required cell lines. Finally, it was found that in in vivo experiments, the artificially constructed GSTO1 Knockout (KO) cell lines exhibited growth delay and even failed to form tumors; while in in vitro experiments, the 3D spheroids formed by these cell lines were smaller compared with normal cell lines [6].

Multi-omics analysis of GSTO1 KO cells revealed that their growth was strongly positively correlated with cell adhesion molecules and interferon response pathways, and strongly negatively correlated with the Myc transcriptional signature. In addition, several clinically used drugs showed significant synthetic lethality in the absence or inhibition of GSTO1 [6]. The transcriptional and protein expression levels of tissue factor (gene name: F3) were downregulated in response to GSTO1 KO. F3 is associated with poor patient survival and promotion of tumor progression in various cancers, and is a known risk factor for metastasis. The transcription of F3 is regulated by IL1β, and the secretion of IL1β is reduced under the inhibition of GSTO1, suggesting that IL1β links GSTO1 expression and F3 transcription. In conclusion, the research results indicate that GSTO1 is indeed a potential therapeutic target for cancer. Figure 3 reveals the various relative indicators of the KO group.

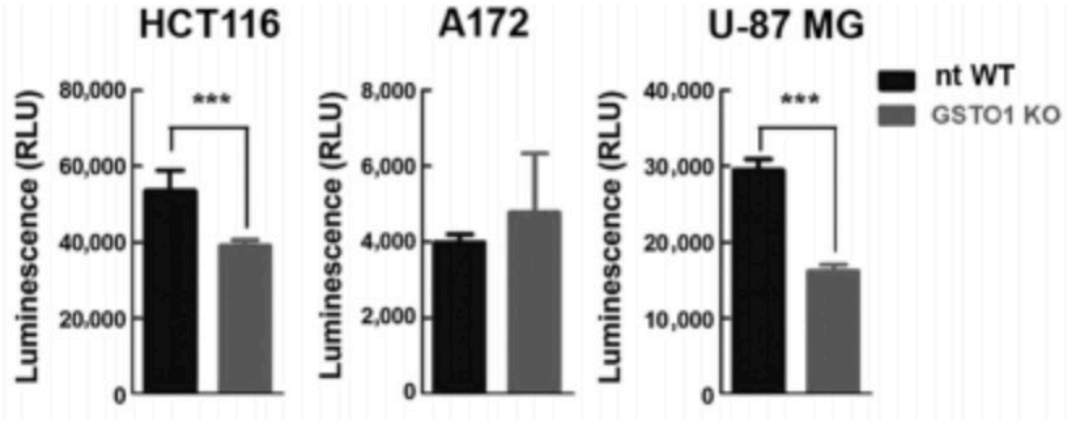

Figure 3. Various indicators of the GSTO1 KO group

3. Analysis of lysine-targeted covalent inhibitors for GSTO1

3.1. Characteristics and analysis of covalent inhibitors

Covalent small-molecule drugs have advantages such as high selectivity, long-lasting efficacy, and improved therapeutic effects on drug-resistant diseases. Covalent inhibitors irreversibly bind to target protein residues through covalent bonds, altering the spatial structure of the target protein, thereby inhibiting the activity of the protein. Small-molecule drugs first bind to the target protein through reversible non-covalent interactions, and then the warhead binds to the active site of the protein through covalent bonds.

The formation of this covalent bond is irreversible; therefore, covalent small-molecule drugs possess the characteristics of high biochemical efficiency, with strong and long-lasting effects. However, if the warhead has the risk of off-target binding, it will lead to significant side effects. This is because such non-selective binding will affect the function of normal proteins, and the impact of this covalent bond is irreversible, thus resulting in high toxicity. Balancing the reactivity and stability of the covalent warhead is a key focus and challenge in the development of covalent drugs.

3.2. Reasons for choosing lysine targeting

Lysine has an exposed side-chain amino group, making it a potential candidate site for the development of covalent ligands. As one of the most common amino acids in the proteome, lysine is widely distributed on the surface of proteins and is involved in Protein-Protein Interactions (PPI) as well as in the functionally active pockets inside proteins. Although lysine has relatively low intrinsic nucleophilicity (pKa = 10.5), its pKa value may be perturbed by the microenvironment, which may enhance its reactivity towards covalent modification.

However, the current problem lies in the lack of covalent warheads targeting weakly nucleophilic amino acids, and the warhead structures targeting tyrosine are single and prone to reaction promiscuity. Therefore, conducting research on covalent warheads targeting weakly nucleophilic amino acids such as tyrosine is beneficial for expanding the library of covalent warheads and providing more options for the development of covalent inhibitors.

Currently, DNA-Encoded Chemical Libraries (DEL) have been widely used in drug discovery in both academic and industrial fields. Incorporating electrophilic warheads into DEL to generate covalent DNA-Encoded Chemical Libraries (CoDEL) can facilitate the screening of covalent inhibitors without relying on the structure of known ligands, thereby offering unique advantages [7]. Large-scale CoDEL has been proven effective in identifying cysteine-targeted covalent inhibitors for a variety of protein targets. Covalent DNA-Encoded Chemical Libraries (CoDEL) represent an advanced technology in covalent drug discovery. A method for identifying lysine-targeted covalent inhibitors using proteome-wide data-guided CoDEL selection has also been discovered. In this case, a strategy can be introduced that combines proteome-wide reactive lysine data from Activity-Based Protein Profiling (ABPP) technology with CoDEL coupled to various covalent warheads, providing a generalizable workflow for the discovery of lysine-targeted covalent inhibitors.

3.3. Evaluation of research results on lysine-targeted inhibitors

Among numerous covalent inhibitors, the development and application of C1-27 and its derivatives deserve the most attention [8]. Based on the reactivity of cysteine in GSTO1, researchers decided to use the α-chloroacetamide scaffold as a warhead to bind to GSTO1. Among them, the work of the Neamati research group is particularly prominent. They conducted a detailed search and screening of a library of compounds containing the α-chloroacetamide structure, and finally discovered and identified C1-27 as a potent GSTO1 inhibitor (half maximal inhibitory concentration IC_{50} = 31 nM), which can react with the C32 site to form a covalent conjugate. Encouraged by the covalent inhibitory property of C1-27, researchers developed a C1-27 derivative with a BODIPY (boron dipyrromethene) group and used it for proteome labeling. Experimental results showed that C1-27 has very good selectivity for GSTO1, maintaining selectivity even at concentrations up to 1 μM. However, it only exhibits weak affinity or reactivity towards Protein Disulfide Isomerase (PDI). Subsequently, researchers evaluated C1-27 using several cancer cell lines (such as HCT116 and HT29 cell lines). These evaluation results showed that C1-27 has a half maximal Growth Inhibitory Concentration (GI_{50}) of 1.2-4.3 μM, indicating that C1-27 can inhibit cancer cell proliferation. Moreover, C1-27, at concentrations of 0.2-3 μM, has the ability to inhibit the colony formation of HCT116 cells.In addition, researchers also tested the in vivo efficacy of C1-27 in xenograft models constructed from colon cancer cell lines. The results showed that when C1-27 was administered at concentrations ranging from 25-45 mg/kg, the high-concentration group (45 mg/kg) significantly inhibited tumor growth without cases such as toxicity and weight loss.

Although C1-27 exhibits nanomolar-level inhibitory activity at the molecular level, it has weak cell inhibitory activity (micromolar level) and a short in vivo half-life (several minutes to one hour), which greatly limits its clinical application. According to the literature, the inhibitory activity of C1-27 at the molecular level does not depend on the originally designed

sulfonamide warhead, but on the chloroacetamide warhead. Therefore, a false positive result was observed at the molecular level. This situation occurs precisely because the amino acid residue matched during the warhead design is the cysteine residue, which is highly reactive and can react with seemingly inactive reaction sites in covalent small molecules, thus highly likely causing off-target issues. Moreover, C1-27 has poor metabolic stability, so no progress has been made in clinical applications. Therefore, it is imperative to search for new targets and synthesize new small molecules. Figure 4 is the schematic diagram of C1-27.

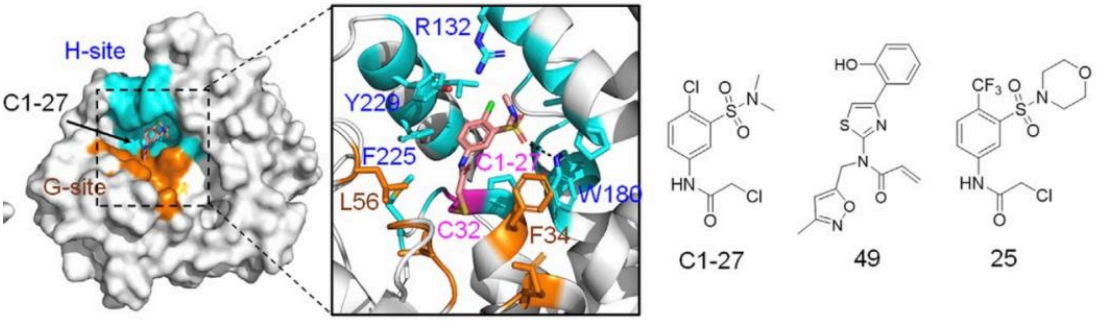

Figure 4. Schematic diagram of C1-27

Fortunately, a research group previously used activity-based proteomics technology and discovered that a class of Phosphoryl Fluoride Warheads (PF-1) can selectively covalently modify the tyrosine residue at position 229 of GSTO1 [9]. Further studies showed that this modification can significantly inhibit the enzymatic catalytic activity of GSTO1, with a mechanism different from that of existing selective GSTO1 inhibitors.

Lysine-Targeted Covalent Inhibitors for GSTO1 will be based on this class of phosphoryl fluoride warheads and employ structure-based drug design, covalent DNA-encoded compound libraries, structural biology, and other methods, aiming to obtain highly active tyrosine-targeted covalent small-molecule inhibitors for GSTO1. The advantage of such small-molecule inhibitors lies in their excellent medium stability and plasma stability, featuring high stability and long-lasting effects.

4. Conclusion

This paper primarily explores the necessity and principles of research on tyrosine-targeted covalent inhibitors of Glutathione Transferase Omega 1 (GSTO1), with a focus on introducing the mechanism of action of GSTO1 and its related covalent inhibitors, and briefly explaining the rationality of selecting tyrosine as a target. It can be concluded that choosing tyrosine residues as targets is indeed pharmacologically rational, and the newly discovered PF-1-type phosphofluoride warhead does exhibit inhibitory activity. However, the article only discusses PF-1 as the phosphofluoride-type covalent inhibitor and does not mention other covalent inhibitors of this type. In future research on lysine-targeted inhibitors for GSTO1, more attention should be paid to the stability of small-molecule drugs, as this characteristic often determines that the reactivity of such drugs is not very strong. Hence, efforts should be focused on modifying the surrounding groups of the drug to maximize the enhancement of non-covalent interactions between the small molecule and the vicinity of the tyrosine residue at position 229 of GSTO1, improve the reactivity between the modified drug and the tyrosine residue of GSTO1, and strive to achieve nanomolar-level reaction activity.

References

[1] Oppong, D., Schiff, W., Shivamadhu, M. C., & Ahn, Y. H. (2023). Chemistry and biology of enzymes in protein glutathionylation. *Current opinion in chemical biology, 75*, 102326. https: //doi.org/10.1016/j.cbpa.2023.102326

[2] Board, P. G., Coggan, M., Chelvanayagam, G., Easteal, S., Jermiin, L. S., Schulte, G. K., Danley, D. E., Hoth, L. R., Griffor, M. C., Kamath, A. V., Rosner, M. H., Chrunyk, B. A., Perregaux, D. E., Gabel, C. A., Geoghegan, K. F., & Pandit, J. (2000). Identification, characterization, and crystal structure of the Omega class glutathione transferases. *The Journal of biological chemistry, 275*(32), 24798–24806. https: //doi.org/10.1074/jbc.M001706200

[3] Whitbread, A. K., Masoumi, A., Tetlow, N., Schmuck, E., Coggan, M., & Board, P. G. (2005). Characterization of the omega class of glutathione transferases. *Methods in enzymology, 401*, 78–99. https: //doi.org/10.1016/S0076-6879(05)01005-0

[4] Shen, N. X., Luo, M. Y., Gu, W. M., Gong, M., Lei, H. M., Bi, L., Wang, C., Zhang, M. C., Zhuang, G., Xu, L., Zhu, L., Chen, H. Z., & Shen, Y. (2024). GSTO1 aggravates EGFR-TKIs resistance and tumor metastasis via deglutathionylation of NPM1 in lung adenocarcinoma. *Oncogene, 43*(33), 2504–2516. https: //doi.org/10.1038/s41388-024-03096-z

[5] Dong, J., Mao, J., Wu, W., Qian, X., & Yu, Z. (2025). FTO Suppresses Proliferation and Induces Apoptosis of T98G Glioblastoma Cells via N6-methyladenosine Modification of GSTO1. *Neurochemical research, 50*(2), 83. https: //doi.org/10.1007/s11064-025-04334-w

[6] Xu, Y., Bankhead, A., 3rd, Tian, X., Tang, J., Ljungman, M., & Neamati, N. (2020). Deletion of Glutathione S-Transferase Omega 1 Activates Type I Interferon Genes and Downregulates Tissue Factor. *Cancer research, 80*(17), 3692–3705. https: //doi.org/10.1158/0008-

5472.CAN-20-0530

[7] Wu, X., Li, S., Liang, T., Yu, Q., Zhang, Y., Liu, J., Li, K., Liu, Z., Cui, M., Zhao, Y., Han, X., Jin, R., Tan, M., Chen, X. H., Zhao, Y., Zheng, M., Sun, Y., Zhou, L., & Lu, X. (2025). Proteome-Wide Data Guides the Discovery of Lysine-Targeting Covalent Inhibitors Using DNA-Encoded Chemical Libraries. *Angewandte Chemie (International ed. in English), 64*(25), e202505581. https: //doi.org/10.1002/anie.202505581

[8] Xie, Y., Tummala, P., Oakley, A. J., Deora, G. S., Nakano, Y., Rooke, M., Cuellar, M. E., Strasser, J. M., Dahlin, J. L., Walters, M. A., Casarotto, M. G., Board, P. G., & Baell, J. B. (2020). Development of Benzenesulfonamide Derivatives as Potent Glutathione Transferase Omega-1 Inhibitors. *Journal of medicinal chemistry, 63*(6), 2894–2914. https: //doi.org/10.1021/acs.jmedchem.9b01391

[9] Sun, S., Homer, J. A., Smedley, C. J., Cheng, Q. Q., Sharpless, K. B., & Moses, J. E. (2023). Phosphorus fluoride exchange: Multidimensional catalytic click chemistry from phosphorus connective hubs. *Chem, 9*(8), 2128–2143. https: //doi.org/10.1016/j.chempr.2023.05.013